6.6. THE INTERACTION OF COLLEMBOLA AND MYCORRHIZAL ROOTS IN NITROGEN MOBILIZATION IN A SCOTS PINE FOREST SOIL

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INTRODUCTION

The role of soil invertebrates in decomposition processes has received increasing attention in the past decade. Many reports describe net mineralization enhancement by soil animals, though other reports show no such effect or even net immobilization (Witkamp and Frank, 1970; Malone and Reich, 1973; Visser *et al.*, 1981). Some of these inconsistencies may have their cause in different methods e.g., experimental timespan, animal species and density, laboratory or field work. Field experiment differences may be due to differing climatological factors, i.e., evapotranspiration (Meentemeyer and Berg, 1986), environmental conditions and plant species (Swift *et al.*, 1979) and nutrient resource composition (Verhoef and Meintser, this conference).

Microfloral activity readily results in immobilization of elements (Ausmus *et al.*, 1976). The interaction between fauna and microflora may, therefore, be crucial for net mineralization. It can be postulated that the microflora in general, and mycorrhizal plant roots as a particular group (considered a mineral sink in the soil ecosystem) may obscure faunal effects on mineral element mobilization, and possibly on other decomposition parameters like the rate of litter weight loss.

Thus, during experimentation it would be necessary to describe fungal or mycorrhizal activity. Since manipulating microfloral activity as a variable is nearly impossible, we have concentrated on manipulation of mycorrhizal tree roots. By preventing mineral uptake by the tree roots we expected to maximize detectability of faunal effects on mineral element mobilization.

An untreated forest floor was compared with one where plant roots were excluded. In a long-term experiment a selected group of fungal grazers were studied for their effect on decomposition processes. Particular emphasis was given to nitrogen mineralization and mobilization. In this paper some data obtained in the early period of this experiment will be presented.

<center>MATERIAL AND METHODS</center>

Site description

The experimental site is located in a 30-year-old pine forest plantation (*Pinus nigra* Arnold subsp. *nigra*) without understorey vegetation. The forest is situated in the north-eastern periphery of Zuid-Oost Flevoland, The Netherlands, at latitude 52°34' N and longitude 5°47'E, on recently reclaimed polderland with a sandy soil. Many mollusc shells are scattered throughout the soil profile; soil pH is 6.4. The mean annual air temperature is 8.5°C, with a maximum of +27.5°C in July and a minimum of -17.0°C in January. Throughfall precipitation during the experiment measured between 560 and 740 mm yr^{-1}. Mean highest groundwater level is 0.80 metre.

Decomposition and nitrogen mineralization as affected by two collembolan species, *Orchesella cincta* (L.) and *Tomocerus minor* (Lubbock) were studied in comparison with an "absent" soil fauna (referred to as "control"). Other faunal treatments also included, will not be presented here. In half the plots tree roots were cut, and therefore, absent. Every combination of treatment levels was replicated four times. The experimental parameters described in this paper are loss of dry weight of organic matter in litterbags, changes in nitrogen content of the organic matter, and mobilized mineral nitrogen (established through collection of leachate in a vacuum tube lysimeter).

Enclosure plots, measuring 0.5×1.0 m, were constructed of stainless steel plates driven into the ground vertically to a depth of 15 cm (Fig. 1). Tree roots were cut and permanently excluded from the exclosures by plates to a depth of 1.1 metres. This was done in December 1985, nine months before the start of the experiment. Animals were prevented from entering or leaving the plots by strips of glue ("Bird Repellent", Rentokil Ltd., Felcourt, East Grinstead, Sussex, England) on both sides of the plates, as well as by a gauze net supported by PVC tubes arching over the plots.

Orchesella cincta and *Tomocerus minor*, both being dominant species at the field site, were collected by hand sieving. Adults only were selected. Animals were introduced into the defaunated litter in August 1986 at "natural" densities (unpublished data), i.e. 2000 collembolans per sq m. Soil burrowing or flying invertebrates could not be prevented from entering plots, but were removed repeatedly by a dry flotation technique (Ernsting, 1988).

Immediately before construction of the plots, the organic material (A_o) was removed, and separated into litter (L), fermentation layer material (F), and humus (H). All materials were dried at 40°C, homogenized, and stored for six months in the laboratory. Twigs and cones etc., were removed. The humus was then sieved over a 0.35 mm gauze to segregate smaller particles which were spread out in the plots evenly. The A_o-horizons were laid down in June 1985:3100 g dry wt of 'H' humus (CN = 22.8; organic matter = 86%), 2100 g dry wt of 'F' material (C:N = 32.7), and 500 g dry wt of 'L' litter (C:N = 57.8) per sq m (including litterbag contents): equalling natural amounts at this site.

The litterbags measuring 10×10 cm (100% polyester) had differing mesh sizes and initial dry weights: 75 g of 'H' humus was confined in a mesh bag with 350 µm gauze bottom side and a 1.0 mm gauze top side; 25 g of 'F' material was put in a 2 mm mesh bag, and 11 g of 'L' litter in 4 mm mesh bag. These mesh sizes were chosen to minimize losses of material through handling, while minimizing possible restrictions of mobility for animals that are endemic to the particular layer. Losses of material due to handling were accounted for by correction of the initial weight. Litterbags were put out in July 1986 and sampled in January 1987. Water content of the litter did not significantly differ between
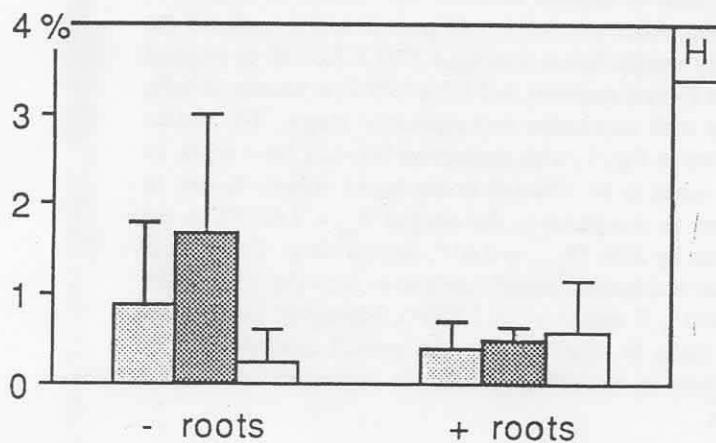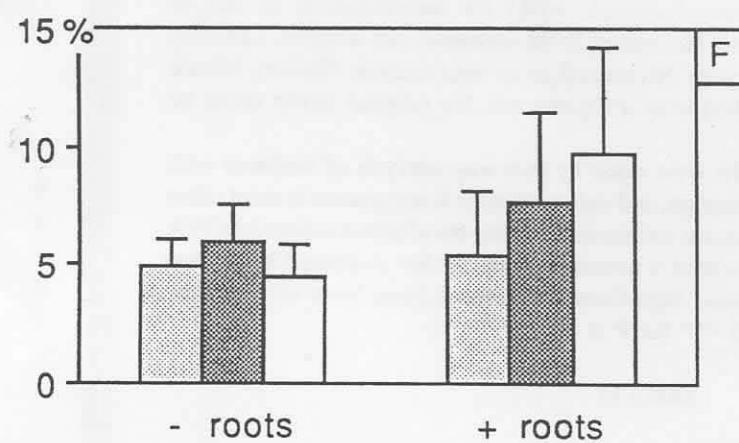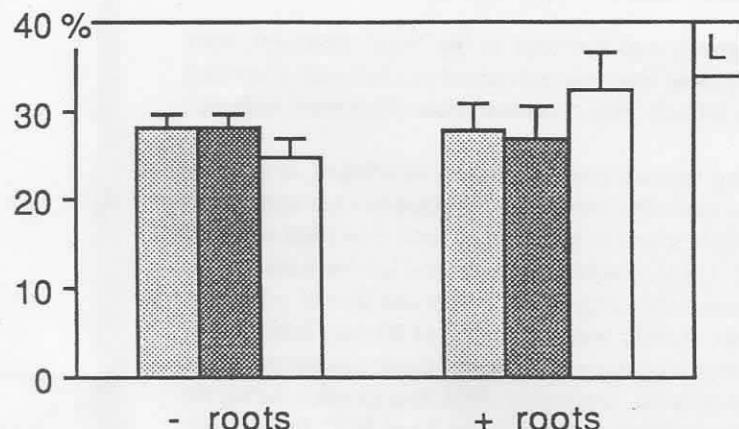
Fig. 1. Weight loss in 'L', 'F' and 'H' horizons over a period of six months after introduction of animals in percentage of initial dry weight (mean ± S.D.). Note different scales on the abscissa. Medium bars: *Orchesella cincta*; dark bars: *Tomocerus minor*; white bars: control.

root treatments. Tree roots that had grown into the bags in the 'root' treatment, were subtracted from weight measurements, and the litter was pulverized in a ball mill. Elemental carbon and nitrogen were measured on a Carlo Erba Strumentazione Elemental Analyser, model 1106.

Litter leachates were collected using vacuum tube lysimeters, installed at an angle of 15° and set at 5 cm depth (topside) in the sand. The vacuum tube lysimeters were constructed of PVC tubes, Ø4.5 cm, 35 cm in length, glued to a porous ceramic cup (Soil moisture Equipment Corp., Santa Barbara, Calif., USA), placed in the plots and left for three months to equilibrate to soil nutrient composition, while sucking in at least one litre of soil water. At approximately three-month intervals, suction was established at 80cbars initial under pressure, and samples were collected over a nine-day period. In this paper data are presented for the first sampling after introduction of fauna, November 1986. Precipitation during the suction period was 30 mm, ambient air temperature ranged between 3 and 10°C. Immediately upon collection, leachates were divided in two, and one half acidified to pH $\leq$ 2 with concentrated nitric acid (Baker Instra-Analysed, 70%) for determination of NH_4-N contents. NO_3-N contents and pH were determined in the untreated sub samples. Leachate concentrations of NO_3-N and NH_4-N were determined on an auto analyser (Skalar). Nitrate was measured as nitrite after reduction over a Cd-column. No original nitrite could be detected.

The overall analyses of the results were done by two-way analysis of variance with replication. Data showed normal distribution, and variances were homogenous in most cases (Bartlett). Data that showed inhomogenous variances, were log-transformed before ANOVA testing. Differences between levels within a treatment were further evaluated by a t-test of the differences between two means. Significant differences have been indicated by *for $P \leq 0.05$, ** for $P \leq 0.01$, and *** for $P \leq 0.001$.

RESULTS

Decomposition rate

Decomposition (meaning weight loss) of organic material was studied in relation to two collembolan species and tree roots. After a seven-month period, which included the summer at autumn season, overall dry weight losses averaged 27.0 ± 3.07 % of original dry weight in the litter, 6.6 ± 3.39 % in F-layer material, and 1.2 ± 1.89 % in humus; clearly, the coefficient of variation increases with successive decomposition stages. The results for the individual treatments are shown in Fig. 1, with distinction between litter types. In a rootless environment, weight loss tends to be retarded in the upper organic layers: in litter, tree roots enhanced weight loss as compared to the control ($t_{[6]}$ = 3.432*), in the F-layer tree roots enhance weight loss by 50% ($F_{s1,23}$ = 4.61*, overall data). Collembola enhance decomposition slightly in litter and humus, significantly so in litter (by 13%) when roots are absent (O. cincta: $t_{[6]}$ = 2.441*, T. minor: $t_{[6]}$ = 2.589*). Moreover, in the litter the interaction between roots and fauna is significant in the overall analysis ($F_{s1,5}$ = 6.12**), which may be interpreted here as: the effect of fauna is dependent on absence or presence of roots, and vice versa.

Changes in total nitrogen

Net gains or losses of total nitrogen in organic material were determined in relation to the occurrence of tree roots and the collembolan species *Orchesella cincta* and *Tomocerus*

minor (Fig. 2). The overall data show a slight net increase of 2.6 ± 73.16 µmol N/g drw in litter, and decreases of total nitrogen in 'F' (42.3 ± 89.61) and humus (9.4 ± 25.51). The high variance in the data is largely caused by individual recordings, within a treatment, contrasting in sign. The F-layer had a net loss of nitrogen in seven months in the presence of roots, showing no change or a minor net gain when absent: thus, tree roots determine the direction of the nitrogen flux ($F_{s1,23} = 23.84***$).

The effect of Collembola could not be distinguished from the control except in humus material ($F_{s5,23} = 2.621*$). Here, in the absence of tree roots, *Orchesella* caused a net decrease of total nitrogen by nearly a three-fold over the control ($t_{[6]} = 4.158**$). *Tomocerus* had no effect.

Leaching of mineral nitrogen

In November 1986, three months after addition of animals, the influence of tree roots and Collembola was studied on the amount of mineral nitrogen in the leachate (Fig. 3). No NO_2-N could be detected. Nitrate dominated by a 100-fold over ammonium as the form of mineral nitrogen in the leachate. Overall NO_3-N and NH_4-N concentrations averaged 0.6 ± 0.34 mmol NO_3 L^{-1} and 6.1 ± 2.62 µmol NH_4 L^{-1} respectively. In untreated plots about twice the amount of NO_3-N was detected in the leachate, when compared to root treatments ($F_{s1,23} = 11.514**$), suggesting uptake of nitrate-nitrogen by the roots. No such difference was observed for NH_4-N ($F_{s1,23} = 0.283$ NS).

Collembola showed no effect on leaching of NH_4-N ($F_{s2,23} = 1.242$ NS), but in the absence of tree roots, *Tomocerus minor* caused 50% more leaching of NO_3-N than the control without fauna ($t_{[6]} = -3.516*$).

<div align="center">DISCUSSION</div>

In the present research decomposition and nitrogen mobilization in *Pinus nigra* litter have been studied under field conditions. Tree roots were excluded from experimental plots to minimize any concealment of collembolan mediated mineralization of nitrogen by mycorrhizal root uptake of nitrogen compounds. In the experimental design, fungal immobilization of nitrogen was not interfered with. In a recent field study, variable levels of microbial (with exception of mycorrhiza) activity was proved to influence the effects of soil fauna on decomposition processes (Teuben, this conference). Furthermore, at this moment we do not know how much the cutting of tree roots in our experiment has shifted relative abundances of different microbial groups. However, with these restrictions born in mind, our results substantiate to the postulated masking of animal mediated mobilized nutrients by rapid microbial uptake, as is discussed here.

The effect of Collembola on the decomposition rate in the litter and F layers in the presence of mycorrhizal tree roots seems to be a retardation, whereas the tree roots alone enhance litter mass loss. Contrarily, grazing on saprotrophic non-mycorrhizal fungi only (in rootless plots) enhances decomposition rate, but these rates equal those found in presence of roots. To explain this, two assumptions are needed. Firstly, the stimulative action of roots on decomposer activity of the mycorrhiza may be diminished by the animals through grazing of mycorrhizal hyphae. Secondly, Collembola may show a grazing preference for mycorrhizal fungi (Shaw, 1985), possibly attracted to them by fungal odours (Bengtsson et al., 1988). It would appear then that mycorrhizal fungi are more sensitive to collembolan grazing than non-mycorrhizal fungi, at least in this time of year. Though the phenomenon
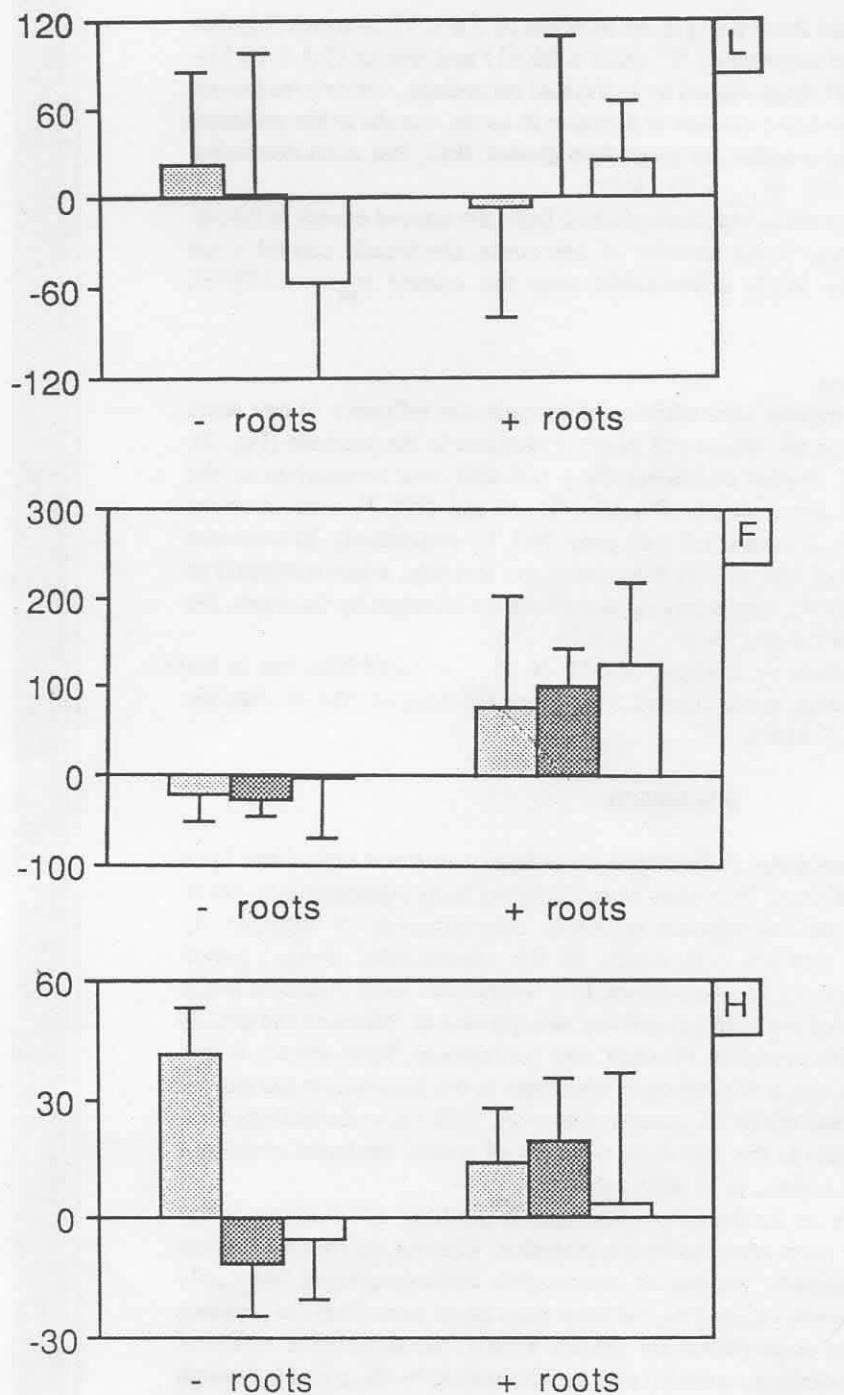
Fig. 2. Changes in the amount of total nitrogen in 'L', 'F', and 'H' horizons over a period of six months after introduction of animals (mean ± S.D., in μmol N g^{-1} dry wt.). Note different scales on the abscissa. Medium bars: *Orchesella cincta*; dark bars: *Tomocerus minor*; white bars: control. N.B.: positive values indicate a loss of total nitrogen.
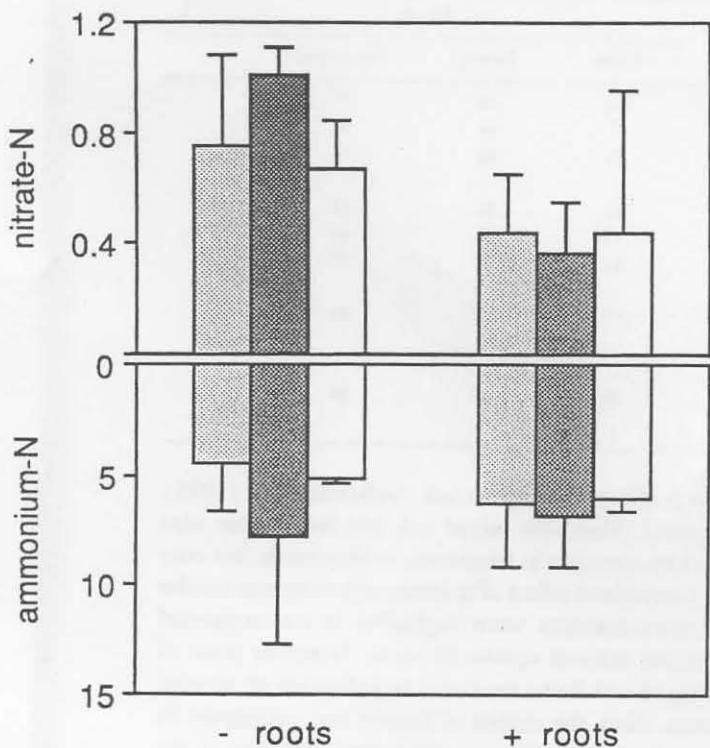
Fig. 3. Mineral nitrogen contents in the leachate, three months after introduction of animals (mean ± S.D.). Note different values and dimensions on the abscissa: nitrate-N in mmol L^{-1}, ammonium-N in μmol L^{-1}. Medium bars: *Orchesella cincta*; dark bars: *Tomocerus minor*; white bars: control.

is not significant in this case, the tendency to a decline in mycorrhizal effect on litter weight loss described here is in agreement with earlier reports of collembolan grazing on mycorrhizas causing less nutrient uptake and retarded plant growth (Warnock *et al.*, 1982; Finlay, 1985). In general, in rooted plots an increase of decomposition rate was observed, coupled with an evident loss of total nitrogen. The most important result on decomposition rate in this study is the significant interaction between the "root" treatment and the "fauna" treatment in litter (Table 1). A second significant interaction between these treatments was found for changes in the amount of total nitrogen in the humus layer (Table 1). In both cases the interactions are interpreted as significant effects of fauna on the respective parameters, occurring only in the absence of roots. The mechanism behind these interactions may be a rapid absorption of mineral elements, that become available through animal action by the mycorrhizal root system. Depletion of available nutrients by tree roots is suggested by the data on leaching of mineral nitrogen. However, it remains to be demonstrated that indeed mycorrhizal tree root activity (and microbial activity in general) causes this interaction, since our results only relate to the presence of roots rather than root activity.

In contrast to Ineson *et al.* (1982), who described an enhancement of leaching of ammonium-nitrogen as a consequence of fungal grazing by Collembola in microcosms

Table 1. Summary of overall effects of experimental treatments on experimental parameters, as tested by ANOVA

Parameter	Soil horizon	Factor		
		Roots	Fauna	Interaction
Decomposition rate	L	ns	ns	**
	F	*	ns	ns
	H	ns	ns	ns
Total nitrogen	L	ns	ns	ns
	F	***	ns	ns
	H	ns	ns	**
Nitrate-N in leachate	A	**	ns	ns
Ammonium N in leachate	A	ns	ns	ns

containing oak litter, in this study no such effects were detected. Anderson *et al.* (1985), reporting on a field study using microcosms filled with mixed oak and beech litter, also described the highest leaching of ammonium-nitrogen in treatments with animals, but only in the absence of roots. In leaf litter (F), a combined effect of animals and roots was similar to the control. In this litter, losses of nitrate-nitrogen were negligible. It was suggested by these authors, that animals may facilitate mineral uptake by roots. From our point of view, their findings illustrate the masking of soil fauna mediated mobilization of mineral nitrogen through the uptake by tree roots. Also, the results of Ineson and colleagues fit to this view, since the reported increase in the leaching of ammonium-nitrogen in the presence of Collembola only occurred after fungal standing crop had dropped significantly in the animal treatment.

The results of the present study explain the contradictory findings observed earlier since seasonal aspects may be of influence for the outcome of the interaction between fungivores and mycorrhizal tree roots (see below), and hence, condition the interaction between microflora and soil fauna. It appears that some of the faunal effects described are specific to collembolan species, as well as to separate organic horizons or resource qualities. This puts matters into an intricate perspective, which is not further discussed here. Further research is going on, focussing on closely related collembolan species and their specific effects on decomposition processes. Others have reported on the consequences of nutritional quality of litter for collembolan-mediated mobilization of minerals (Verhoef and Meintser, this volume).

SUMMARY

The effect of soil dwelling Collembola on nitrogen mobilization was studied experimentally in a plantation of *Pinus nigra*. After the removal of all litter dwelling soil fauna, *Orchesella cincta* and *Tomocerus minor* were kept in enclosures. A control with no soil fauna was also set up. Tree roots were cut and permanently excluded from half of the enclosures; as a result, these plots lack the presence of mycorrhizal mycelia. It is postulated that the microflora in general, and mycorrhizal plant roots as a particular group, may obscure faunal

effects on mineral element mobilization, by the rapid uptake of available nutrients. Over a six-month period, the decomposition rate of litter, F-layer, and humus in litterbags, changes in the total nitrogen content of these litter types, and the mineral nitrogen content of the leachate were described. It is concluded that Collembola can significantly influence the rate of weight loss in litter, as well as amounts of total nitrogen and leacheable mineral nitrogen. These effects could only be established by the exclusion of tree roots.

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